

Continuous *Moniezia benedeni* Infection in Confined Cattle Possibly Maintained by an Intermediate Host on the Farm

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ABSTRACT. Infection with *Moniezia benedeni* is sometimes found in confined cattle in Japan. Between October 2011 and January 2013, we monitored the fecal egg prevalence at a confined cattle farm in Miyazaki prefecture where continuous *M. benedeni* infection has been recognized for years to evaluate the possible infection routes. Fecal egg prevalence changed seasonally with the highest in October 2011 (27.3%: 9/33). This was followed by a gradual decrease until July 2012 (9.4%: 3/32) and then an increase between August to December 2012 when new egg-excreting cases were observed. The pattern of seasonal changes was similar to that reported previously for cattle kept in a barn with an outside playing yard. Although *M. benedeni*-infected mites were not found, we constantly detected an oribatid mite, *Oribatula sakamorii* Aoki, 1970, in the litter of cattle bedding from May to October 2012. This species belongs to a genus which has been reported to be a suitable intermediate host for *M. benedeni*, suggesting that *M. benedeni* infection may have been autonomously maintained at the farm via oribatid mites living in the cowshed. When infected cattle were treated with praziquantel, it was found that a single oral inoculation with a dose of 5 mg/kg was effective for deworming.

KEY WORDS: confined cattle, epidemiology, *Moniezia benedeni*, *Oribatula sakamorii*, treatment.

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Moniezia benedeni is a cestode which infests the small intestine of ruminants, such as cattle, sheep and goats. The parasite is distributed worldwide and is especially prevalent among grazing animals, but rarely found among confined animals [8]. Although *Moniezia* spp. are distributed widely in Japan, the number of cattle infection studies is limited, and most studies involve pasture grazing cattle and those kept in barns with an outside playing yard [5, 7, 9–11, 16]. The parasite needs an intermediate host, the oribatid mite, to complete its lifecycle. There are approximately 170 families, 1,200 genera and 9,000 valid species and subspecies of oribatid mites that live in soil and litter [15]. Of these, only 37 species were reported to serve as the intermediate host of *M. benedeni* [4]. In Japan, over 1,000 species have been reported [3], including susceptible genera for *Moniezia*, such as *Oribatula* and *Scheloribates* [13]. Actual routes of transmission between cattle and mites, however, have not been

clearly defined.

It is generally considered that the pathogenicity of *M. benedeni* is low, because the host does not show specific or serious symptoms. However, loss of growth efficacy has been recognized in infected calves [10]. In addition, some farmers claim that calves that expel large proglottids have reduced value at auction. Therefore, worm control is beneficial, even though pathogenicity is low. It was reported that administration of 3.75 mg/kg of praziquantel (PZQ) was effective for complete deworming of sheep [14] and that 5.0 mg/kg was effective for goats [12]. However, an effective dose of PZQ in cattle for *M. benedeni* deworming has not yet been clarified.

In 2011, we encountered a breeding farm suffering from continuous *M. benedeni* infection in confined cattle for many years. In this study, we tried to clarify the reason for the continuous *Moniezia* infection at the farm by evaluating the possible existence of the intermediate host in the roughage provided to the cattle, the surrounding environment of the farm and the litter at the farm. In addition, we tried to determine a suitable dose of PZQ for the treatment of infected cattle.

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Table 1. Monthly egg-prevalence in breeding cows and the number of new cases starting excretion of *Moniezia benedeni* eggs in cows and calves

		2011			2012								2013		
		Oct.	Nov.	Dec.	Jan.	Mar.	Apr.	May	July	Aug.	Sep.	Oct.	Nov.	Dec.	Jan.
Cows	No. surveyed	33	33	32	32	32	31	32	32	32	32	32	32	32	32
	No. egg-positive	9	8	5	6	6	5	4	3	4	4	5	5	4	4
	Prevalence	27.3	24.2	15.6	18.8	18.8	16.1	12.5	9.4	12.5	12.5	15.6	15.6	12.5	12.5
	No. new case	–	1	0	0	0	0	0	0	1	0	1	1	0	0
Calves *	No. surveyed	27	28	20	23	20	16	14	14	11	12	17	15	19	18
	No. new case	–	3	3	0	0	0	0	1	0	0	2	1	5	0

* Egg-prevalence in calves was not evaluated, since infected calves were treated immediately.

MATERIALS AND METHODS

Study farm: The farm was located in Miyazaki, Japan and had raised about 30 Japanese black breeding cows and their calves under 8 months old by a loose housing free barn system since 2007. From the beginning, the cattle were kept in a cowshed and never grazed. In March 2011, fecal egg examination of all cattle at the farm revealed *M. benedeni* infection in 19.4% of breeding cows (7/36) and in 26.1% of calves (6/23). All infected cattle were treated with PZQ (10 mg/kg), and five days after the treatment, all cattle were negative for *M. benedeni* eggs. However, in September 2011, fecal egg examination revealed that *M. benedeni* was again present.

Sample collection: Following the recurrence of *M. benedeni* infection, we monitored the change in egg-prevalence of breeding cows and also checked new cases for egg excretion in breeding cows and calves. Stool samples (rectal feces) were collected from all cattle (31–33 breeding cows and 11–28 calves) once a month from October 2011 to January 2013, except for February and June 2012. Samples were stored at 4°C and examined within a month of collection.

Fecal egg examination: A modified Wisconsin sucrose centrifugal flotation technique [6] was conducted using 10 g of feces, and eggs were identified morphologically.

PZQ treatment in calves: When *M. benedeni* eggs were detected in calves, PZQ was administered at three different doses of 10, 5 and 1 mg/kg body weight (BW). The effect of PZQ was evaluated by monitoring egg excretion from the calves after treatment. Fecal samples were collected once or several times between 0 to 5 days before and several times between 1 to 7 days after the administration of the anthelmintic. Fecal samples were stored at 4°C, and the parasite eggs were examined as described above.

Collection of epidemiological information: To elucidate the factors contributing to *M. benedeni* endemicity at the farm, the following information was obtained. 1) The original farm from which the cattle were introduced and the status of *M. benedeni* infection at the original farm, 2) The environment around the farm, 3) The frequency of weed elimination around the cowshed, 4) Disinfection of the environment surrounding the farm, 5) The disposal process for litter and muck in the cattle bedding and 6) Type, source and management of roughage as cattle feed.

Collection of oribatid mites: In order to determine whether oribatid mites, the intermediate host species for *M. benedeni*,

were present at the farm, mites were collected from organic matter, such as roughage, residues in feed trough and litter in cattle bedding in December 2011, January 2012 and monthly from May to November 2012. On each sampling day, 1–3 kg samples were collected and examined. In addition, plastic dishes of feces containing *M. benedeni* eggs were set as bait traps for oribatid mites and placed on the bedding in the cowshed for one week. Seven bait traps were set at least 5 m apart from each other. Oribatid mites and other soil animals were collected from the samples directly into 70% ethanol using Tullgren funnels with 25 W bulbs for 72 hr. The morphology of the collected oribatid mites was observed under a Nomarski's differential interference microscope (NIKON Eclipse E-600, Nikon, Tokyo, Japan) after the specimens were mounted on slide glasses with gum chloral, and species were identified according to the method described by Aoki [1, 3].

RESULTS

During the study period, the breeding cows at the farm were relatively fixed, except for two cows which were moved out from the farm individually in December 2011 and April 2012, and one calf that was born on the farm and incorporated into the breeding group in May 2012. Monthly egg prevalence in the breeding cows during the study period is shown in Table 1. The highest egg prevalence was found in October 2011 (27.3%), and the lowest was in July 2012 (9.4%). Two cows continually excreted the eggs throughout the study period. New cases of *M. benedeni* egg excretion were found mainly between October and December and to a lesser degree in July and August 2012 (Table 1). Mean numbers of parasite eggs per gram of feces (EPG) in breeding cows varied from 0.2 to 1,079.0 and in calves from 0.2 to 1,368.6. There were no regular fluctuations in EPG according to the season or duration of infection. Clinical symptoms relating to *M. benedeni* infection, such as bowel movement disorders or diarrhea, were not recognized.

The following information was obtained relating to the factors contributing to the endemicity of *M. benedeni*. 1) Cattle were originally introduced from one farm where *Moniezia* infection was prevalent. 2) The study farm was located between a paved road and a wooded hill, and there was no grassland near the farm. 3) The area surrounding the farm was well managed by cutting weeds as necessary and

Table 2. The number of oribatid mites detected from different organic matters in the study farm

Year	2011	2012						
Month	Dec.	Jan.	May	July	Aug.	Sep.	Oct.	Nov.
No. mites detected	1	None	1	2	1	5	1	None
Developmental stage of mite ^{a)} × No.	F × 1		Larva × 1	M × 1, F × 1	F × 1	M × 4, F × 1	M × 1	
Type of biomass sample ^{b)}	R		L	L	B	L	L	

a) M: adult male, F: adult female, attached number: number of mites. b) R: residues in feed trough, L: litter in cattle bedding, B: bait trap.

by disinfection with quicklime. 4) Litter in cattle bedding was scraped out at least once in the summer season and once a month during the winter season and composted with good fermentation. 5) The cattle were fed mainly with wrapped rice-straw silage produced on-site. 6) However, from May to July 2012, the cattle received a supplementary feed of Italian ryegrass silage produced by the other farm from which the cattle were originally introduced. *Moniezia* infection had been prevalent for a long time, and the farmer distributed unfermented cattle muck to the grass fields at this farm.

From the organic matters, soil animals, such as flour mites, *Laelapidae* mites, collembolan and soil nematodes, were detected throughout the study period. These soil animals were detected more often from old litter than from new litter. Oribatid mites were also continuously detected, especially in the litter from cattle bedding, but not from compost and roughage (Table 2). All detected oribatid mites were identified as *Oribatula sakamorii* Aoki, 1970 [1] (Fig. 1). A larva of this mite was also found in May 2012. In addition, *Oribatula* sp. was detected from rectal feces of a cow at the egg examination performed in November 2012, but species identification was not possible due to physical damage.

Changes in the number of EPG in infected cattle after PZQ administration at different doses are shown in Table 3. Before administration, infected cattle excreted the eggs continuously. Following treatment with doses of 10 or 5 mg/kg BW, the mean EPG was reduced by 99.4 and 99.7% at day 1 post-administration and by 100% at days 5 and 7 post-administration, respectively. On the other hand, the mean EPG was reduced by only 56.1% at 5 days post-administration of 1 mg/kg BW PZQ.

DISCUSSION

Most of the studies on bovine *Moniezia* infection in Japan have been exclusively focused on pasture grazing animals or those kept in a barn with an outside playing yard, and there are few reports for confined cattle [5, 7, 10, 16]. This study farm had raised cattle without grazing and had suffered from a long-term *Moniezia* infection. The seasonal change in egg prevalence among the cattle showed a similar pattern to that reported in cattle kept in a barn with an outside playing yard [10]. Since the prevalence was highest in autumn and lowest in early summer, it was suspected that it was affected by the availability of the intermediate host.

From the epidemiological information obtained, the following is suspected. 1) The cattle of the farm were initially introduced from a *Moniezia*-endemic farm, and thus, they

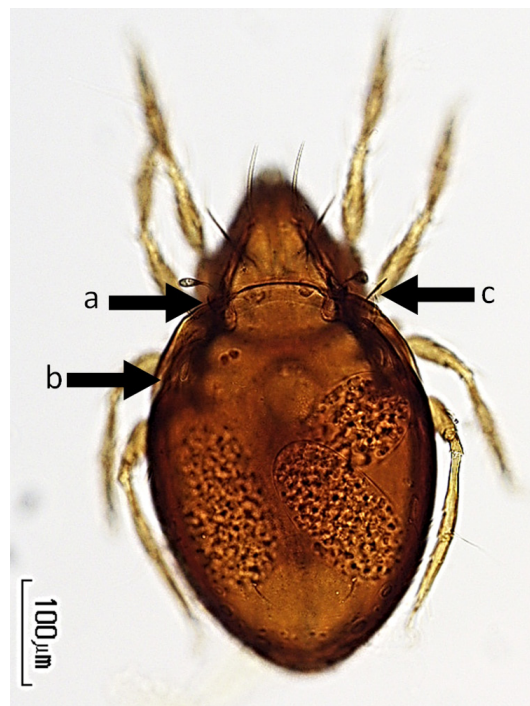


Fig. 1. *Oribatula sakamorii* detected from the litter in cattle bedding of the study farm. (a) sensillus, (b) areae porosae and (c) notogastral seta in the bothridium and its vicinity show the typical morphological features of the species.

carried *M. benedeni*. 2) Considering the environmental situation around the farm and the production system of compost and roughage, it seems unlikely that there was any incidental invasion of infected oribatid mites from the surrounding environment or home-produced roughage. However, the cattle received a supplementary feed of roughage provided by a *Moniezia*-endemic farm. *Moniezia benedeni* infection via home-produced grass has already been reported in Japan [9]. Considering that *M. benedeni*-infected oribatid mites could be present in the roughage provided, further investigations to find *Moniezia*-infected mites in the roughage are needed to fully evaluate this risk.

At the study farm, however, the silage from the *Moniezia*-endemic farm was fed to the cattle only during May to July of 2012. Considering that the prepatent period for *M. benedeni* is about 40 days, new cases of egg excretion found between July and August are possibly explained by the silage. How-

Table 3. Monitoring of *Moniezia benedeni* egg-excretion before and after praziquantel (PZQ) administration with different doses

A. PZQ 10 mg/kg bw (n=15)

	Before administration	After administration (day)		
		1	3	5
No. egg-excreting cattle	15	6	2	0
Mean EPG	198.2	1.2	0.1	0
Reduction rate (%)	–	99.4	99.9	100

B. PZQ 5 mg/kg bw (n=7)

	Before administration	After administration (day)			
		1	3	5	7
No. egg-excreting cattle	7	6	1	1	0
Mean EPG	767.6	2.5	0.2	0.2	0
Reduction rate (%)	–	99.7	100.0	100.0	100

C. PZQ 1 mg/kg bw (n=2)

	Before administration	After administration (day)		
		1	3	5
No. egg-excreting cattle	2	2	2	2
Mean EPG	1,185.5	887.8	1,031.7	520.1
Reduction rate (%)	–	25.1	13.0	56.1

EPG: the number of eggs per gram of feces.

ever, new cases were also detected from October 2012 to December 2013, and thus, it was suspected that another source of infection could have existed at the study farm.

To evaluate the possibility that the intermediate hosts were present at the farm, we collected oribatid mites from the farm's organic matter. We detected oribatid mites continuously, especially from the cattle bedding litter. Oribatid mites have been found previously at an outside playing yard for cattle in Japan [17], but this is the first report of the mites inhabiting cattle bedding inside the barn. The detected mites were identified as *O. sakamorii*, the genus of which has been reported to be an intermediate host of *M. benedeni* [4]. This mite species has been reported to live in the soil containing low biomass, such as those at farming fields, sandbreak plantations along beaches and greenbelts of urban area [2], suggesting the mites can live in cattle bedding. Since a larva of the mite species was also detected, it was suspected that the mites were maintained in the cattle bedding at the farm. Moreover, mites belonging to the same genus were detected in the rectal feces of a cow, suggesting that cattle at the farm had actually ingested the mites. Infection of cattle by ingestion of oribatid mites living at the farm can be confirmed, if an infected mite is found in the litter of the farm. It was also suspected that a low frequency of exchange of cattle bedding, especially in summer, would provide a good habitat for the mite.

Our results suggested that even confined cattle can be infected with *Moniezia* sp. via infected oribatid mites in the roughage or living in the cattle bedding litter. The latter route of transmission indicates that once *Moniezia* sp. is introduced to a farm, the infection can be autonomously maintained.

Administration of PZQ at 5 mg/kg BW resulted in complete elimination of *M. benedeni*. This dose in cattle is comparable to that in sheep and goats [12, 14]. Although the dose of PZQ at 1 mg/kg BW did not completely eliminate the parasites in this study, evaluation of doses >1 and <5 mg/kg BW would be worthwhile (e.g. 2.5 mg/kg BW). Considering that the prevalence was highest and further transmission seemed to cease in winter, treatment of the cattle and complete replacement of litter in winter would be highly effective for eliminating *M. benedeni* from endemic farms. To avoid further introduction of *M. benedeni* infection, it is also important to have a quarantine system for newly introduced cattle.

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